FREQUENCY DISTRIBUTION OF HOST-RANGE MUTANTS AMONG T2 PHAGE INFECTED BACTERIA

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Luria (1951) has developed an interesting theory of the mode of replication of bacteriophage genes based on evidence from the study of frequency distributions of plaque type mutants (r and w types) among T2L phage infected bacteria.

The present study was undertaken in view of a possible generalization of his theory with respect to the host range mutation.

MATERIALS AND METHODS

Phage. A plaque type mutant phage, T2 r tu, was used as a test bacteriophage. Its latent period and burst size, when incubated with Escherichia coli strain B cells in nutrient broth at 37°C, were about 20 min and 50, respectively.

Host bacteria. Escherichia coli strain B was used as host. To select for host-range mutants, strain B/2 which is resistant to T2 was used.

Techniques. Standard methods in phage work described by Adams (1950) were adopted. For basic medium, nutrient broth (pH 7.2) was used. Incubations were carried out at 37°C throughout the experiments. The details of the other experimental procedures will be described later.

RESULTS

Host-range mutation in T2 bacteriophage. The host range mutants (h mutants) are characterized by their ability to attack the phage resistant strain, and they have been found only in T2 in the T-even series.

The first quantitative and systematic analysis of the h mutation was made by Luria (1945), and the subject has been developed by Hershey et al. (1948, 1949, and 1951) with the T2H strain. The main features of the host range mutations in the T2H strain are the following: The host range mutants can be classified into several categories in terms of the ratio of the plaque counts on B/2 to the plaque counts on B. This ratio is called the efficiency of plating (e.o.p.); it is a heritable characteristic of the virus, and varies between 0.01 or less and unity for different mutants. These h mutants are also distinguishable by their plaque morphology. Mutants with e.o.p. near unity form clear plaques (phenotype h°) on the indicator strain B/2. Mutants with e.o.p. of less than 0.3 form turbid plaques (phenotype h') and, the lower the e.o.p., the more turbid the plaques.

The most important feature is that the h mutations occur at two different loci. In our phage strain this was confirmed as follows: Crosses between any two of the 10 independently arising mutant strains of phenotype h° failed to yield recombinants. However, in crosses between an h° mutant whose e.o.p. was 0.1 and any one of the above 10 h° mutants, about 3 per cent of wild type phages were always found among the phage progeny. This indicates that the h° mutation occurs at a single locus and the h' mutation at some other locus, as shown by Hershey and Davidson (1951). The frequency of the h° mutants was about 10^{-7} and that of h' mutants was between 10^{-4} and 10^{-5} in our T2 strain.

Frequency distribution of h° mutant clones among individual bacteria. The frequency distribution of the host range mutants of phenotype h° among the phage infected bacteria was investigated by the so-called single burst technique with some modifications.

Procedure. Actively growing cells of E. coli strain B were centrifuged and resuspended in fresh broth to about 2 \times 10^6 cells per ml. The washed cells were infected with T2 r tu phage in such amounts that each bacterium was infected, on the average, with two or three phage particles. After adsorption for 5 min, during which about 80 per cent of the phage input was adsorbed, the cell phage mixture was washed three times at

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1 This study was reported at the International Genetics Symposia held at Tokyo in September 1956. This study was supported by a grant for scientific research from the Department of Education of Japan.
0 C in order to eliminate the unadsorbed free phages. Then the cell suspension was diluted 1:50 into fresh broth and aliquots of 0.2 ml were distributed into series of many tubes. Consequently each tube contained about $8 \times 10^4$ cells. These were incubated in a water bath at 37 C for 30 min (the total incubation period, therefore, was 35 min). When the incubation time was over, these tubes were heated at 58 C for 20 min, stopping the further development of phage growth in order to avoid a possible second growth cycle of the newly formed phages. Then the whole content of each tube was assayed on each plate with the indicator B/2, on which only the $h$ mutant phages can form plaques.

In every experiment, some large $h$ mutant clones were found which were probably due to the bursts of bacteria infected with the $h^c$ mutant phages already present in the input phage. In the present study, all $h$ mutant clones larger than 30 were considered as derived from mutants present in the input phage. This decision seemed reasonable, since the frequency of such clones was approximately equal to the expected frequency calculated from the amount of $h^c$ mutants in the added phage stock, and since the

**TABLE 1**

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Mutant Bursts per Plate</th>
<th>Mutant Clone Size per Plate</th>
<th>Full Mutant Clone, 30 or More</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.375</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>3c</td>
<td>0.20</td>
<td>1</td>
<td>(2)</td>
</tr>
<tr>
<td>4a</td>
<td>0.445</td>
<td>2</td>
<td>(4)</td>
</tr>
<tr>
<td>4b</td>
<td>0.22</td>
<td>2</td>
<td>(1)</td>
</tr>
<tr>
<td>4c</td>
<td>0.031</td>
<td>1</td>
<td>(3)</td>
</tr>
<tr>
<td>5a</td>
<td>0.17</td>
<td>1</td>
<td>(1)</td>
</tr>
<tr>
<td>5b</td>
<td>0.16</td>
<td>1</td>
<td>(1)</td>
</tr>
<tr>
<td>Total frequency</td>
<td>38</td>
<td>9</td>
<td>2 (11)</td>
</tr>
<tr>
<td>Cumulative frequency</td>
<td>73</td>
<td>35</td>
<td>32</td>
</tr>
</tbody>
</table>

**Figure 1.** The distribution of T2 $h^c$ mutant clones among individual plates.

burst size of T2 r tu under the experimental conditions was about 30.

The corrected distributions of the mutant clones per individual plate are shown in table 1. Here, it is observed that the small sized clones appear relatively frequently, and the larger the clone size, the lower the frequency.

According to the data in table 1, 275 $h^c$ mutant plaques were found among $1.6 \times 10^5$ original particles distributed among 456 plates. The average rate of $h^c$ mutants, excepting the large size clones, was $1.73 \times 10^{-2}$, and the average number per plate was 0.6. Accordingly, if these mutant particles were distributed at random among the assay plates, 151 plates would contain one mutant, 45 plates would contain 2, 9 plates would contain 3, and only 2 plates would contain more than 4 mutant particles. It is thus clear that the distribution of the $h^c$ mutant plaques is not random but clonal.
DISCUSSION

Luria (1951) has pointed out that the increase in number of the genetic determinants of a virus particle will be exponential in time if each replica acts as a source of new replicas. Assuming a constant probability of mutation per individual replication, therefore, mutant phage will appear clonally in individual bacteria with a frequency inversely proportional to the number of mutants. Hence, there are the following relations between the frequency of mutant clones and their sizes:

\[ Y_x = \frac{2mN}{x}, \quad (N \gg 1), \]

where \( Y_x \) indicates the cumulative frequency of mutant clones larger than \( x \), \( x = \) the mutant clone size, \( m = \) the mutation rate, and \( N = \) the final number of replicas or the burst size.

If so, then it follows that when log \( Y_x \) is plotted versus log \( x \), all points will be located on a straight line at an angle of minus 45 degrees which passes through a point corresponding to \( x = 1 \).

Now, let us examine whether the data obtained fit Luria’s hypothesis or not. In figure 1, the abscissa indicates the mutant clone size, \( x \), and the ordinate the cumulative frequency of the mutant clones larger than \( x \). Both are plotted on a logarithmic scale.

As may be seen in this figure, there is a fairly good agreement, within the limits of experimental error, between the theoretical expectation and the data obtained. Here it must be noted that if the phenomenon of “phenotypic mixture” discovered by Hershey et al. (1951) will occur in such bacteria in which \( h \) mutation had occurred, plaque counts of the \( h^c \) mutants obtained by plating directly on B/2 will be necessarily low. However, the lowering of that e.o.p. of the \( h^c \) mutant does not seem to affect remarkably the shape of the curve for the mutant distribution.

The author has reported a similar result obtained with another coli bacteriophage, P6 strain, with respect to the host range mutation (Tanami, 1955). It is apparent that the distribution of the \( h^c \) mutant clones among individual bacteria is of an exponential type, indicating that the replication of the virus genes proceeds as expected from Luria’s theory with respect to the host-range mutation, too. This will suggest that the “vegetative” phage particles of Visconti and Delbrück (1953) may multiply at an exponential rate chiefly by successive duplications.

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SUMMARY

The frequency distribution of the host-range mutant phages of T2, phenotype \( h^c \), among individual bacteria was investigated for the purpose of analyzing the mutation process and reproduction rate of intracellular virus particles.

The distribution of the mutant clones with respect to their size was clearly an exponential type, indicating that the replication of the virus genes proceeds in such a way that replicas themselves may serve as patterns for additional replications.

REFERENCES


LURIA, S. E. 1945 Mutation of bacterial viruses affecting their host-range. Genetics, 30, 84-99.

